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EFFECT OF INTERFERON ON VIREMIA DURING ARBORVIRUS INFECTION

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13. ABSTRACT

This paper presents the results of viremia in mice experimentally infected with the Venezuelan equine ensephalomyelitis virus. It is shown that the greater acceptability to infection with this virus is expressed in a higher degree of viremia. It is established that the greater levels of viremia coincide with maximum content of interferon in the blood. In absolute terms, the production of interferon in suckling mice was lower than in mature mice, although titer of virus in the blood os sucklings was considerably higher than in adult mice. In this paper, the conditions for the effects of interferon on neurovirus infection in the viremia phase are considered.

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FOREIGN TITLE: VLIYANIE INTERFERONA NA VIRUSEMIYA PRI ARBOVIRUSNOJ INFEKTSII

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NOTICE

Viremia is present in the majority of virus infections. At the same time, its intensity and duration vary in different virus infections. Viremia takes place as the result of the emergence of the virus from the cell and its appearance in vascular channels. It has been established that the viremia appears to be the principle pathogenic symptom of arborvirus transmitted by the sting of arthropods.

The invasion of the macroorganism by a virus stimulates a specific immunologic response and also some complex nonspecific reactions. The production of interferon is related to one of these nonspecific reactions of the organism to viral infection [10].

We know that newborn mice show a higher degree of sensitivity to infection with various arboviruses than mature mice. This raises the question of whether the increased sensitivity of newborn mice is related to their lower ability to form interferon. The purpose of our investigation is the study of the characteristics of the development of viremia in mice, both adults and sucklings, under conditions of experimental infection by Venezuelan equine esephalomyelitis (VEV), the determination of interferon in the serum of infected animals and the study of the effect of interferon on viremia.

Material and Method

Viruses. The VEV virus was used for the production of experimental infection in mice after several passages through mouse brain. During the course of this experiment, passages were carried out in suckling mice infected with a 10^5 delution of the virus. The titer of the virus was determined in cultures of chicken fibroblasts by the plaque method. To obtain control preparations of interferon, we use the New Castle virus (NV). The Indiana strain of the virus of vesicular stomatitis was used to titrate the interferon as to the quality of the indicator virus. All viruses were received from the virus museum of the D. I. Ivanovskiy Institute of Virusology of the Academy of Medical Sciences of the USSR.

Infection of Animals and Extraction of Blood. In these investigations, we used non-pedigreed white mice weighing 18-20 g and 2-3 day old sucklings from non-pedigreed white mice. In the adult animals, the brain was injected with up to 0.04 ml and the sucklings received up to 0.02 ml of the virus suspension.

Blood was taken from the adult mice by puncture of the retro-orbital venous channels described by Chan Shi-Tsze and V. V. Pogodin [5]. In sucklings, the blood was obtained with a sterile pipette from vessels dissected through an incision in the anterior chest wall.

Tissue Cultures and Their Infection. In the experiments for the study of viremia, the virus was titrated on initially trypsinide cells of chicken embryos. Trypsonization of 9-10 day old chicken embryos was carried out by the commonly accepted method. The cells were grown in culture dishes with a 50 ml capacity, into which was placed up to 10 ml of a cell suspension in a concentration of $1.5 \cdot 10^6$ cells per ml. The growth medium consisted of 0.5% hydrolysate of lactalbumin in Hanks' solution with 10% normal bovine serum, 100 units of penicillin and 100 mg of streptomycin per ml of medium. The culture dishes were incubated at

37°C. A compact single layer of cells was formed over a period of 48 hours. Prior to infection, we decanted the culture medium, washed the cells once with Hanks solution and the remaining culture medium was removed with a pipette. After infection (up to 0.2 ml of the infecting material was placed in each culture dish) and after incubation for one half hour at 37°C with subsequent removal of unabsorbed virus, the cells were covered with agar according to the directions given by L. G. Karpovich [2], in which, instead of the serum from newborn calves, recommended by the author for the culture of group B arborviruses, the normal bovine theorum was used. The plaques were counted in 48 hours.

A transplanted culture of L-cells was used in titrating interferon. The L-cells were separated from the glass by means of 0.25% solution of trypsin warmed to 37°C. After 5 minutes of contact with the cells with the trypsin solution and its removal to the culture dishes, the medium was added in which the cells were suspended. The resulting cell suspension was planted in test tubes up to 200,000 cells per ml, and the later were incubated at 37°C. The formation of the cellular layer of the necessary quality took place in 24 hours.

Acquisition and Titration of Interferon. The source of the tested interferon was the serum obtained at various intervals following infection from mice infected with VEV virus. The control preparations of interferon were obtained from mice weighing 18-20 g who received intrevenous injections of up to 1 ml of NV cultures having 10^7-10^8 MLD_{50}. These mice were exsanguinated in four hours, since it has been shown that in this period of time interferon appears regularly in a sufficiently high titer [3]. To free the serum of virus, it was processed with a 0.1 M solution of HCl solution at a pH of 2.0. After keeping at 4° for four hours, alkalinization was carried out with a solution of sodium bicarbonate to pH equals 7.2-7.4. The preparations showed a complete absence of virus activity.

The material containing interferon was titrated in the test tube cultures of L-cells. The experiment was carried out with the control (serum of normal mice not containing interferon, and serum collected from mice 4 hours after their infection with NV containing interferon in a titer of 1:1,024). Prior to the beginning of the experiment, the culture solution was decanted and up to 1 ml of 2 dilutions of interferon in medium No. 199 was added to the test tube. The contact between interferon and L-cell lasted 18 hours at 37°C. After this the interferon was decanted and we added 100 MLD_{50} vesicular stomatitis virus contained in 1 ml of medium No. 199. This dose of virus causes a complete destruction of the cell layer in 48 hours. Interferon titer was accepted to be that maximum solution of serum which suppressed the action of vesicular stomatitis virus in half of the infected test tubes.

Results

In experiments for the study of viremia in mice whose brains were infected with a dose of virus containing $4 \cdot 10^4$ BOE/ml, accumulation of virus was determined in the blood at various intervals from 2 minutes to 120 hours. In each established interval, a group of 10 animals was used. From Table 1 it may be seen that 2 minutes after intracerebral injection of virus in suckling white mice there was already viremia of $7.9 \cdot 10^3$ BOE/ml. In the course of the first 3 hours following infection, the concentration of virus in the blood of suckling mice decreases very slowly and reaches $5 \cdot 10^2$ BOE/ml in 3 hours. After this, viremia again increases at which time 2 waves of increased viremia clearly appear. The first wave of increased concentration of virus in the blood coincides with a point in time 4-6 hours after infection of suckling mice and the second begins 18 hours after infection of the animals. The viremia in the suckling mice is maximum at 30-48 hours, i.e., corresponding to $2.5 \cdot 10^9$-$3 \cdot 10^{11}$ BOE/ml. During this period, these animals show the symptom complex of experimental ensephalomyelitis and they begin to die. In adult mice, as we can see from Table 1, the virus does not occur in the blood in the course of the first 4 hours. The virus begins to appear in the blood channels 5 hours after infection, and in 6 hours it circulates in the blood at a low titer of $1 \cdot 10^2$ BOE/ml. In 18 hours viremia occurs in $2.5 \cdot 10^5$ BOE/ml and after this the concentration of virus in the blood gradually increases, reaching a maximum figure in 24 hours of $1.75 \cdot 10^3$ BOE/ml. This level of viremia remains until 48 hours after infection. After this, the titer of virus from the blood gradually decreases and in the course of 72 hours becomes $1.52 \cdot 10^6$ BOE/ml, and in 120 hours becomes 4.10^3 BOE/ml. The first signs of infection appeap in 48 hours: the mice are somewhat lifeless , their fur is disheveled. In 72 hours, the symptoms increase: lifelessness increases and paresis and paralysis appear. The animals begin to die during the period of clinical ensephalytis. The maximum period of survival of an adult mice was 99-120 hours after the moment of infection.

Interferon (Table 2) appears in the serum of suckling mice 6 hours after infection and gradually increases in titer. Thirty hours after infection the titer of interferon in the serum of sucklings is maximum -- 1:128. In mature mice, the production of interferon began 10 hours after infection and gradually increased. Thirty hours after infection the titer of interferon in the serum of adult mice reached 1:1.024 and thereafter began to decline. 96 hours after infection of adult mice, there were only residual quantities of interferon; 120 hours later, interferon no longer could be detected in the serum. The relationship between interferon titer and virus appearing in the serum of infected animals is shown in the graph.

TABLE 1

VIREMIA IN WHITE MICE DURING EXPERIMENTAL VENZUELAN ENCEPHALOMYELITIS

Time after infection	Viremia in BOE/ml Sucklings	Adult Mice	Time after infection	Viremia in BOE/ml Sucklings	Adult Mice
2 min.	$7.9 \cdot 10^3$	n.i.	5 hours	$4.2 \cdot 10^5$	50
5 min.	$7.2 \cdot 10^3$	0	6 hours	$3 \cdot 10^6$	$1 \cdot 10^2$
10 min.	$2.9 \cdot 10^3$	0	18 hours	$4 \cdot 10^6$	$2.5 \cdot 10^5$
15 min.	$2.1 \cdot 10^3$	0	24 hours	$8 \cdot 10^7$	$1.75 \cdot 10^7$
30 min.	$2 \cdot 10^3$	0	30 hours	$2.5 \cdot 10^9$	n.i.
1 hour	$1.6 \cdot 10^3$	0	48 hours	$3 \cdot 10^{11}$	$1.43 \cdot 10^7$
2 hours	$1.3 \cdot 10^3$	0	72 hours	n.i.	$1.52 \cdot 10^6$
3 hours	$5 \cdot 10^2$	0	96 hours	n.i.	$3.7 \cdot 10^5$
4 hours	$2.6 \cdot 10^4$	0	120 hours	n.i.	$4 \cdot 10^3$

Note: Here and in Table 2, n.i. equals not investigated; 0 means that the virus was not determined.

Discussion

It is known that the degree of susceptibility of animals to viral infection depends on the age of the host. The greater susceptibility of young animals in comparison with adults appears regularly in arboviruses. There is no unanimity of opinion regarding the mechanism which controls the age-related difference in susceptibility of animals. After the discovery of interferon, the latter itself appeared to be one of the factors which control resistance [9]. Our experiment carried out on animals of various ages, demonstrated a clear difference in the production of interferon by animals of various age groups. In determining the dynamics of the contents of interferon in the serum of infected animals, we observed a correlation between it and the multiplication of the virus. As we can see from the graph, the content of virus in the blood of infected sucklings reached a maximum in 30 hours and in 24-48 hours in mature animals, i.e., in approximately the same period of time following infection, but the intensity of the viremia in these animals differed. More intense viremia developed in sucklings. At the same time, maximum titers of interferon in sucklings and in adults are found at the same time (see graph), i.e., in thirty hours after infection, but the titers of interferon in adult animals were 8 times higher than in sucklings.

It is necessary to note that viremia does not influence the clinical manifestation of infection. The clinical signs of the disease are absent in the animals with high levels of virus in the blood. At the same time, there appear quite high titers of interferon in the serum of these animals. In the case of the suckling mice (see graph) that interferon does not limit the viremia which continues to increase. We did not succeed in following up the ultimate fate of virus and interferon in the bodies of the sucklings, the animals died during the time of a high degree of viremia and considerable titer of interferon was found in the serum.

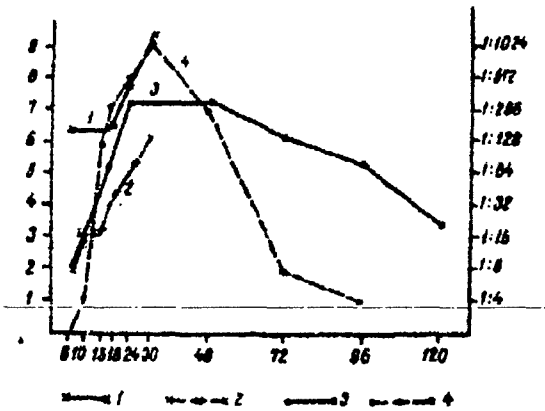
In mature mice there was a different picture. The first signs of illness began to appear when the virus titer of the blood was still not high but the content of interferon was beginning to fall, i.e., 48 hours after infection (see graph). A clear clinical picture of the disease appears 72 hours later, when viremia already shows a distinct tendency to decrease and the interferon is found in lower titers. The pronounced development of the infection and the early demise of suckling mice may be correlated with the less intense production of interferon in the bodies of these animals as compared with mature mice, in addition, there is a lower sensitivity of the suckling mice to the antiviral action of interferon [1].

As regard mature mice, one can propose that interferon to some degree suppresses the reproduction of virus by sensitive cells and for this reason the entry of the virus into the blood is lower.

TABLE 2

PRODUCTION OF INTERFERON IN MICE

Time Blood was taken after Infection	Production of Interferon in the Organism	
	Of Sucklings	Of Adults
6 hours	1:8	0
10 hours	1:16	1:4
15 hours	1:16	1:128
18 hours	1:32	1:256
24 hours	1:64	1:512
30 hours	1:128	1:1024
48 hours	n.i.	1:256
72 hours	n.i.	1:8
96 hours	n.i.	1:4
120 hours	n.i.	0



Relationship Between the Virus Titer and the Interferon in the Serum of Mice.

1 -- Viremia in mouse embryos; 2 -- interferon titers in mouse embryos; 3 -- viremia in mature mice; 4 -- titer of interferon in mature mice. On the abscissa -- time after infection in hours; on the left ordinate -- infectiousness in BOE/ml; on the right ordinate -- titer of interferon.

The causes which contribute to the fatal outcome of the infection in mature mice may be due to the loss of ability of the organism to produce interferon during that period of the infection when antibodies have not yet appeared and when they circulate in a low titer. These data agree with the work of Baron et al [6, 7, 8], V. D. Solov'yeva et al [4].

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